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International application number: PCT/GB05/000174

International filing date: 19 January 2005 (19.01.2005)

Document type: Certified copy of priority document

Document details: Country/Office: GB
Number: 0418141.8
Filing date: 13 August 2004 (13.08.2004)

Date of receipt at the International Bureau: 04 April 2005 (04.04.2005)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)

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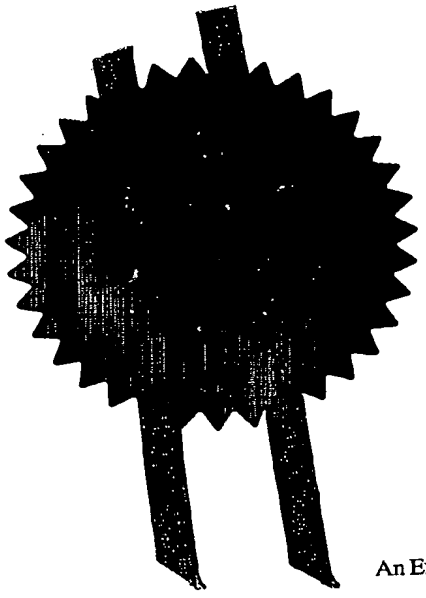

Signed Andrew Gersey

Dated 14 March 2005

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1.	Your reference	IT/RD/N15944
2.	Patent application number *(The Patent Office will fill this part)*	0418141.8
3.	Full name , address and postcode of the or of each applicant *(underline all surnames)*	The School of Pharmacy University of London 29-39 Brunswick Square London WC1N 1AX
	Patents ADP number *(if you know it)*	8928814001
	If the applicant is a corporate body, give the country/state of its incorporation	
4.	Title of the invention	Method of Producing Microparticles
5.	Name of your agent *(if you have one)*	Williams Powell
	"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*	Morley House 26-30 Holborn Viaduct London EC1A 2BP United Kingdom
	Patents ADP number *(if you know it)*	~~830310001~~ 5873031000

6.	If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number	Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*
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8.	Is a statement of inventorship and of right to grant of a patent required in support of this request? *(answer 'Yes if:* *a) any applicant named in part 3 is not an inventor, or* *b) there is an inventor who is not named as an applicant, or* *c) any named applicant is a corporate body*	Yes		

DUPLICATE

Method of Producing Microparticles

The present invention relates to a method of producing microparticles, in particular microparticles of a drug encapsulated by a polymer which allows for drug release in the gastrointestinal tract.

The concept of using pH-sensitive polymers to target drugs to site-specific regions of the gastrointestinal (GI) tract is not a new one. Gastric irritant or labile drugs are routinely administered as enteric coated tablet or pellet systems, and by choosing a polymer with a suitably high dissolution threshold pH, it has been attempted to target the terminal ileum/colon region for the treatment of inflammatory bowel diseases specific to this area.

However, these methods are not without their limitations. The large size of these systems normally results in a delayed gastric emptying, especially when administered after a meal, which will result in a delayed and unpredictable onset of drug action. The GI transit time of large monolithic systems are also subject to more variation than those of multiparticulate systems and this can lead to variation in bioavailability.

The formulation of pH-responsive microparticles using the emulsification/solvent evaporation method may overcome these limitations. Due to their small size, microparticles would be expected to suspend in the gastric contents and therefore empty rapidly through the pylorus in both the fed and fasted state. Transit through the small intestine should be more reproducible, and transit through the colon should be slower, reducing the chances of a colon-targeted dosage form being voided intact. The large surface area of a microparticulate system should also allow a faster drug release once the pH threshold is reached, and as the drug is likely to be dispersed throughout the matrix in an amorphous form, drug dissolution is expected to be more rapid, a particular advantage for drug targeting to the colonic region given the limited fluid volume in this area. Regarding the potential for the colon as a site for protein and peptide delivery,

microencapsulation may be a preferable method of getting such drugs into a delivery system, placing much less mechanical stress on these labile molecules than the preparation of pellets or tablets would.

There are four pH-sensitive Eudragit coatings commonly used as pH-sensitive coatings for tablet, pellet and capsule systems and these are soluble above different threshold pHs; L100-55 (pH 5.5), L100 (6.0), S100 (7.0) and P4135 (7.4). Previous attempts at formulating microparticles of L100 and S100 have been unsuccessful, resulting in particles of poor morphology and control of drug release, and have involved complicated production methods involving homogenisation, careful control of temperature or rate of addition of surfactant.

Given the theoretical advantages of microparticulate systems over conventional dosage forms, the present applicant decided to try to overcome the problems that have led to the production of microparticles of poor morphology and control of drug release. It was decided to optimise the emulsification/solvent evaporation method for the production of Eudragit L/S100 microparticles, a commonly used method of microencapsulation and to apply this optimised method to other MR polymers.

The emulsification/solvent evaporation method is a conceptually simple, three step process. In step one, polymer is dissolved in a suitable solvent (into which the drug is dispersed, or preferentially dissolved), and emulsified into a non-solvent phase usually containing a surfactant to improve emulsion stability. In step two, solvent is allowed to evaporate, usually under agitation. Once this is complete, particles are solidified, and can be separated by filtration and cleaned up. A crucial step in the process for the formation of good particles is the formation of a stable emulsion in the early stages, and to achieve this the choice of surfactant can be considered key. It has also been found that the choice of solvent influences microparticle morphology depending on the rate at which it migrates from the polymer solution into the non-solvent phase and is removed by

evaporation. The solubility of the polymer in the chosen solvent and boiling point are factors that affect how quickly the particles solidify. During this process the forming "particles" will evolve from being liquid emulsion droplets, to semi-solid "sticky" particles, to solidified, discreet particles. The length of time the particles exist in the semi-solid form is expected to influence coalescence of the forming particles and the overall morphology of the end product.

Previous attempts at microencapsulation of Eudragit L100 and S100 have resulted in particles of poor morphology, and a possible explanation for this is the choice of surfactant with aluminium tristearate (Goto et al) and gelatin (Morishita et al) being previously utilised with HLB (hydrophilic-lipophilic balance) values of about 15 and 9.8 respectively.

In accordance with the first aspect of the present invention, there is provided a method of producing microparticles comprising a bioactive and a vehicle, which method comprises providing a solvent having a bioactive dispersed or dissolved therein and a vehicle dissolved therein, carrying out an emulsification to produce an emulsion of microparticles comprising the bioactive and the vehicle in a solvent phase, and evaporating the solvent to leave said microparticles, wherein a mixture of at least two surfactants is employed to stabilise said emulsion and the HLB (hydophilic-lipophilic balance) of the mixture is up to 10.

In a preferred embodiment, sorbitan sesquioleate is used as a surfactant to stabilise said emulsion.

Sorbitan sesquioleate is known by the trade name Arlacel 83 and is an equimolar mixture of sorbitan monoleate and sorbitan dioleate.

The solvent is preferably pure ethanol although a 2:1 mixture of acetone and an alcohol could also be employed.

We believe we have developed a novel emulsification/solvent evaporation method, allowing the fabrication of drug loaded pH-responsive polymeric particles of Eudragit L100-55, L100, S100 and mixtures of the polymers. With subtle manipulation of other parameters, it may also be possible to use the method to microencapsulate other polymers. We have demonstrated the successful microencapsulation of the water insoluble polymers Eudragit RS100 and ethylcellulose.

We have demonstrated the usefulness of Arlacel 83 for the production of Eudragit L100 and S100 particles in particular, and believe our method is superior to other literature methods, in terms of its simplicity and possibility for future scale-up, as well as quality of the final product.

The particles are an ideal size for oral delivery (in the size range 30-50μm) and the excellent morphology should impart good flow properties allowing efficient and reproducible capsule filling. The particles may also be suitable for administration using a buffered suspension.

We have demonstrated a pH-responsive release profile from Eudragit L100-55, L100 and S100 *in-vitro*. Drug release is minimal from all pH-responsive microparticles at gastric pH, but rapid above the threshold of the polymers. A pH-change method has been used to characterise drug release from L100 and S100 microparticles. The drug loading can be manipulated so that less than 10% release occurs after 2 hours in acid, while the time for 100% drug release is less than 5 minutes once pH is raised to intestinal/colonic levels for L100 and S100 microparticles respectively.

The beneficial effect of Arlacel 83 is probably due to an effect on emulsion stability, but this surprisingly does not appear to be due to HLB value alone.

Without wishing to be constrained by theory, one possible explanation is that it is the combination of two or more surfactants (in the case of Arlacel 83, an equimolar combination of sorbitan monoleate and sorbitan dioleate) which functions on a molecular level to stabilise the emulsion. It is thought however that the composite surfactant should still have an HLB in the appropriate range (up to 10, preferably 3 to 6).

We have entrapped a model drug, prednisolone, with good efficiency, but believe the method to be capable of microencapsulating a wide range of pharmaceutical agents. Encapsulation of protein and peptide drugs may also be possible, and these labile drugs are less likely to be deactivated by this formulation method than more traditional tableting or pelletisation methods.

The chemicals used in the process are all widely available, relatively inexpensive and safe. We have shown microencapsulation to be possible using a mixture of organic solvents and, preferably, ethanol alone thus avoiding the use of more toxic solvents. The equipment used in the process is also widely available.

At present no method exists for the large-scale production of Eudragit L100 and S100 particles. Spray-drying has proved unsuccessful due to the thermoplastic nature of the polymers, and its tendency to form stringy aggregates. This leaves the method we have developed as the most feasible alternative.

A number of preferred embodiments of the present invention will now be disclosed, with reference to the following drawings:

Figures 1 to 9 show various scanning electron micrographs (SEMs) of examples and comparative examples of microparticles of drug/polymer mixtures;

Figures 10 to 16 show drug release profiles from various microparticles made in accordance with the invention.

Preliminary experiments using Span 85 as a surfactant were carried out to optimise the choice of solvent mixture. 30mL mixtures of acetone and either ethanol or methanol in different ratios were tried, and it was found that acetone/methanol mixtures worked better than acetone/ethanol, probably due to a faster evaporation of methanol resulting from a lower boiling point and reduced affinity for the polymer, Eudragit S100. When methanol alone was used, large, hollow, and sometimes, cracked particles were produced. Acetone alone did not produce any microparticles. Increasing the proportion of acetone reduced the size but seemed to increase the degree of aggregation. 20mL acetone/10mL methanol was the optimal solvent mixture as judged by SEM analysis, and it was decided to use this in future experiments, and change the surfactant.

It was felt that surfactants with an HLB in the range 1 to 10 would be most suitable for stabilising the emulsion in question. Surfactants in, and close to, this range were therefore sourced, and a simple system using liquid paraffin as non-solvent was tried, with overhead propeller stirring from a Heidolph RZR1 stirrer calibrated to 1000rpm. A mixture of 30mL acetone/methanol (2:1) was used to dissolve 3 grams Eudragit S100 polymer. Stirring and solvent evaporation were allowed to proceed overnight, and the product was collected by vacuum filtration through a sintered glass filter the next day, washed with three 50ml portions of hexane to remove traces of liquid paraffin, and dried in a vacuum oven for 24 hours. All experiments were carried out in triplicate, and the polymer used in the optimisation process was always Eudragit S100.

Span 65 is a cream/yellow solid at room temperature, and was immiscible with liquid paraffin after heating. Furthermore it did not dissolve in the acetone/methanol mixture, and therefore was unable to stabilise the emulsion and produce microparticles.

Comparative Example 2 : Use of oleic acid as surfactant

Next oleic acid was added to the liquid paraffin in concentrations of 1, 2 and 3%.

Oleic acid produced better particles than any surfactant previously used. At concentrations of 1% and 2% w/w particle aggregates were seen. However, the particles comprising the aggregates appeared to be of spherical morphology. At 3% concentration, there was less aggregation, although some small aggregates were still present (see Figs 2A, 2B and 2C). The sample shown is also quite polydisperse. It was concluded that oleic acid stabilises the emulsion to a greater degree than previous surfactants.

Comparative Example 3 : User of Brij surfactants

Two Brijs were then tried with appropriate HLB values; Brij 52 with HLB 5.3 and Brij 92 with HLB 4.9. Brij 52 is a waxy solid at room temperature, Brij 92 is a liquid. Brij 92 was tried at 1, 2 and 3% concentrations.

At 1% Brij 92 concentration, again we see aggregates of semi-formed spherical particles. Increasing the concentration to 2 and 3% does not have a positive influence on microparticle morphology, and the morphology of the 3% sample seems to be the worst of the 3 samples (see Figs. 3A, 3B and 3C).

At room temperature, Brij 52 was a solid and not miscible with liquid paraffin, but upon heating 1% Brij 52 could be dissolved into liquid paraffin and did not precipitate out on

Results: The SEM of the particles is shown below in Fig. 5.

Conclusions: Aggregation is seen in Fig. 5 and is evidence that the beneficial effects of Arlacel 83 in the formation of Eudragit S100 is not due to HLB value alone. It does not appear to be possible to choose a suitable surfactant for an emulsification/solvent evaporation based on HLB value alone. The emuslification/solvent evaporation method is a conceptually simple technique, but Watts et al have proposed that the final structure and composition of a microsphere will result from a complex interplay between polymer, drug, solvent continuous phase and emulsifier. The above results seem to back up this statement.

Example 6

The proposed method of microencapsulation can also be used for Eudragit L100 and mixtures of L100 and S100

Aim: To produce microparticles of Eudragit L100 and mixed Eudragit L/S100 using Arlacel 83.

Method: 3 grams of Eudragit L100 and 3 grams of a 1:1 mixture of Eudragit L100 and S100 were dissolved in 30mL acetone/methanol 1:1 as previously, and emulsified into 200ml liquid paraffin containing 1% Arlacel 83.

Results: On both occasions, particles of excellent morphology were formed, comparable to Eudragit S100 particles (see Fig. 6). The result is not surprising as the only difference between the L100 and S100 polymers is the ratio of carboxylic acid to ester groups, being 2:1 in L100 and 1:1 in S100. This also explains why there is little work in the literature

Results: The SEMs from the above experiments are shown in Figs 7A to 7E.

Conclusions: From the results of the above experiment, various combinations of acetone and either methanol or ethanol have allowed the formation of Eudragit S100 microparticles with excellent morphology. The choice of solvent has little effect on the morphology of the microparticles This provides evidence that the choice of surfactant is more crucial than the choice of polymer solvent. All particles are unaggregated, non-porous and in the desired size range. Preliminary data suggests size of microparticles is influenced by choice of solvent; increasing the proportion of methanol in the solvent mixture seems to increase size, ethanol seems to have the opposite effect. However, it is anticipated that a particle size of less than 500μm is required to negate the effects of the Migrating Myoelectric Complex (MMC) and permit a rapid gastric emptying and all solvent mixtures provided this.

Example 8

Use of ethanol as a sole solvent for the production of microparticles of L100, S100 and L100-55

It would be desirable to produce particles using only ethanol as disperse phase solvent, to simplify the method of production and to reduce toxicity concerns due to any residual solvent in the microparticles, ethanol being less toxic than acetone and methanol. Therefore, 30mL portions of ethanol were used to dissolve 3 grams of L100-55, L100 and S100. The emulsification/solvent evaporation was used as before, with 200ml liquid paraffin containing 1% w/w Arlacel 83 as surfactant. SEMs of the microparticles are shown in Figs. 8A to 8C.

applications, and also show the versatility of our method of microencapsulation, particularly for the Eudragit range of polymers.

Proof of concept: *in-vitro* drug release profiles

Figures 10 to 15 show the following *in-vitro* drug release profiles for microparticles in different pH media, using USPII paddle apparatus. All the microparticles were formed using Arlacel 83 as a surfactant.

Fig. 10 shows prednisolone release from Eudragit L100 (10:1) particles at pH 1.2-6.8. This is an averaged profile of a series of six different examples.

Fig. 11 shows a comparison of prednisolone release from Eudragit S100 microparticles and an equivalent S100 coated tablet system at pH 1.2-7.4.

Fig. 12 is a comparison of prednisolone release from Eudragit S100 microparticles with different drug loadings.

Fig. 13 shows prednisolone release from Eudragit RS/S microparticles (1:1) at pH 1.2-7.4 to demonstrate that water-insoluble Eudragit RS sustains release from S100 particles at colonic pH.

Fig. 14 is a release profile for 6 batches of Eudragit S100/prednisolone (5:1) microparticles at pH 1.2-7.4 which demonstrates batch to batch reproducibility.

Fig. 15 is a profile showing prednisolone release from Eudragit RS/S (1:1) microparticles at gastric pH for 2 hours, proximal intestinal pH for 1 hour, and colonic pH for 2 hours. Little prednisolone release is seen for the first 3 hours, but when the pH is changed to 7.4 the majority of the drug is released over a period of about an hour. This is an averaged profile of four different samples.

Fig. 16 is a release profile comparing RS/S100 (50:50) at pH 1.2-7.4, with ethylcellulose/S100 (50:50). This is essentially comparing the ability of two water insoluble polymers, each in combination with S100, to achieve sustained release profiles. Ethylcellulose seems to perform better, but mixing different proportions of either water insoluble polymer will give different tailored release profiles.

CLAIMS

1. A method of producing microparticles comprising a bioactive and a vehicle, which method comprises providing a solvent having a bioactive dispersed or dissolved therein and a vehicle dissolved therein, carrying out an emulsification to produce an emulsion of microparticles comprising the bioactive and the vehicle in a solvent phase, and evaporating the solvent to leave said microparticles, wherein a mixture of at least two surfactants is employed to stabilise said emulsion and the HLB (hydophilic-lipophilic balance) of the mixture is up to 10.

2. A method as claimed in claim 1, wherein said HLB is from 3 to 6.

3. A method as claimed in claim 1 or 2, wherein said mixture comprises sorbitan monoleate and sorbitan dioleate.

4. A method as claimed in any preceding claim, wherein said mixture is an equimolar mixture of two surfactants.

5. A method as claimed in any preceding claim, wherein the vehicle is a polymer which enables pH-dependent or pH-independent release of the bioactive in the gastrointestinal tract.

6. A method as claimed in any preceding claim, wherein the vehicle is Eudragit ® L100, Eudragit ® L100-55, Eudragit ® S100, Eudragit ® RS100 or ethylcellulose.

7. A method as claimed in any preceding claim, wherein the bioactive is prednisolone.

8. A method as claimed in any preceding claim, wherein the solvent is ethanol or a mixture of acetone and ethanol or methanol.

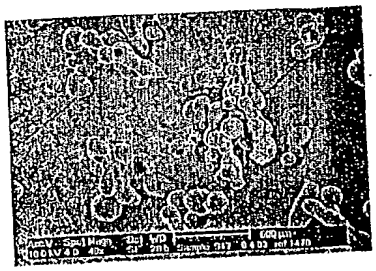


Fig 1: 1% span 85

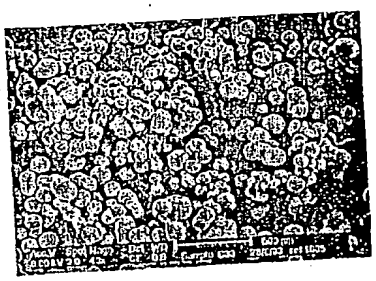


Fig 2A: 1% oleic acid

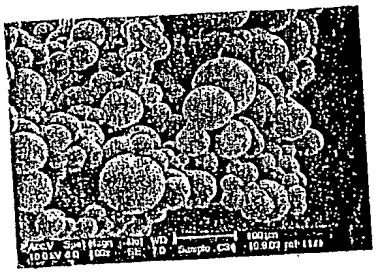


Fig 2B: 2% oleic acid

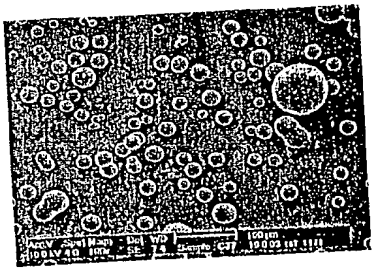


Fig 2C: 3% oleic acid

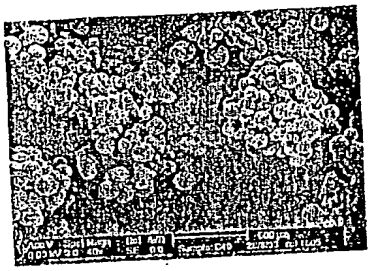


Fig 3A: 1% Brij 92

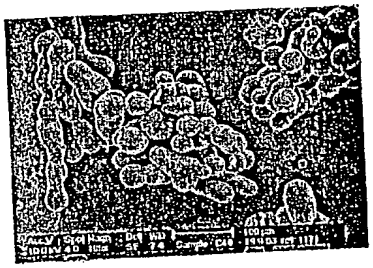


Fig 3B: 2% Brij 92

Fig 3C: 3% Brij 92

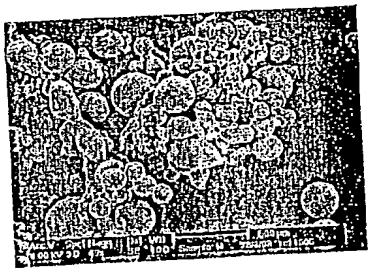


Fig 3D: 1% Brij 52 (dissolved in organic solvent phase)

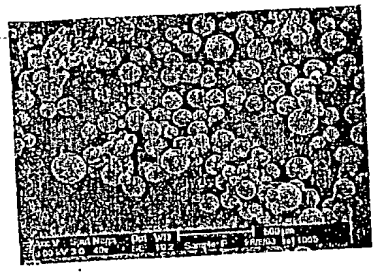


Fig 3E: 1% Brij 52 (heated)

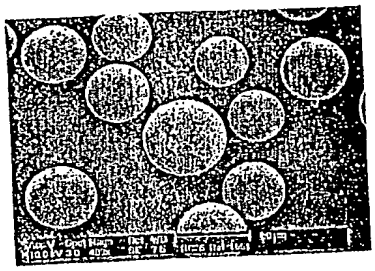


Fig 4A: 1% Arlacel 83

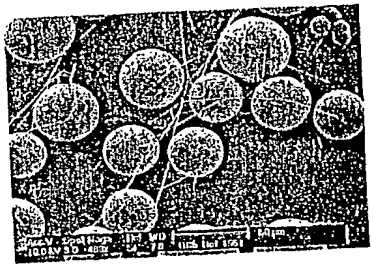


Fig 4B: 2% Arlacel 83

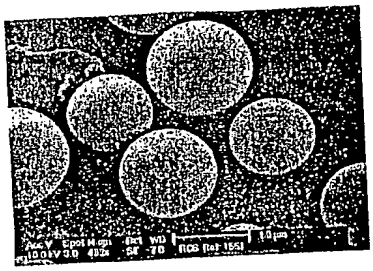


Fig 4C: 3% Arlacel 83

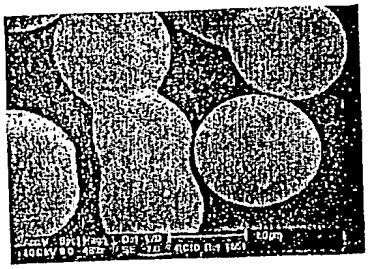


Fig 5: 1% (14.4% Tween 80 and 85.6% span 85)

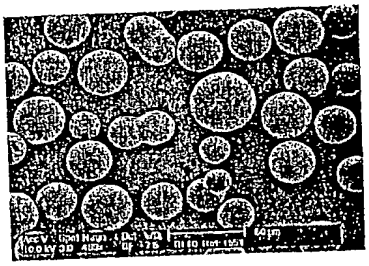


Fig 6: Eudragit L100/1% Arlacel 83/20mL acetone/10mL methanol

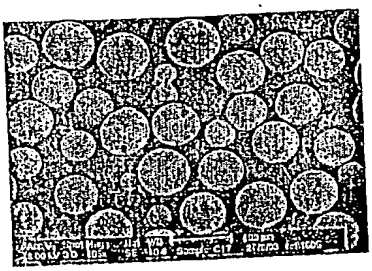


Fig 7A: 15mL acetone/15mL methanol

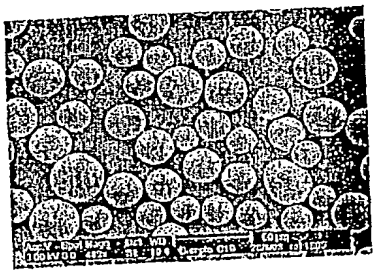


Fig 7B: 15mL acetone/15mL ethanol

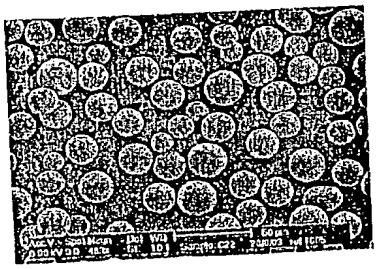


Fig 7C: 20mL acetone/10mL ethanol

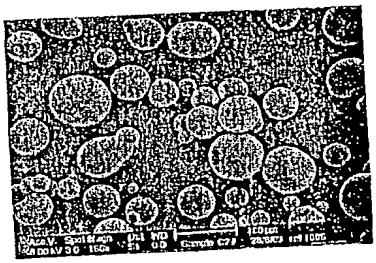


Fig 7D: 25mL acetone/5mL ethanol

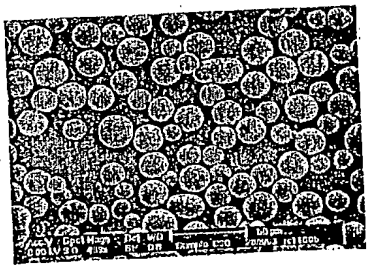


Fig 7E: 25mL acetone/5mL methanol

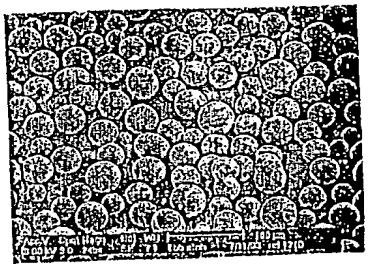


Fig 8A: 3g L100-55/30mL ethanol/1% Arlacel 83

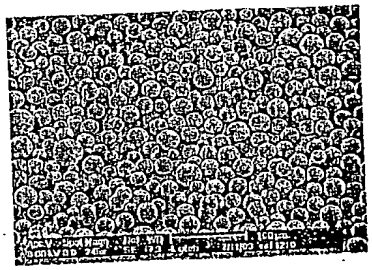


Fig 8B: 3g L100/30mL ethanol/1% Arlacel 83

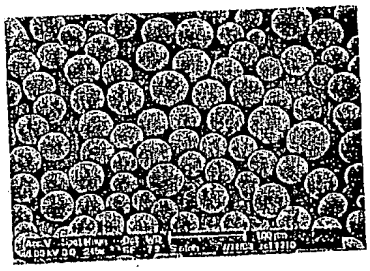


Fig 8C: 3g S100/30mL ethanol/1% Arlacel 83

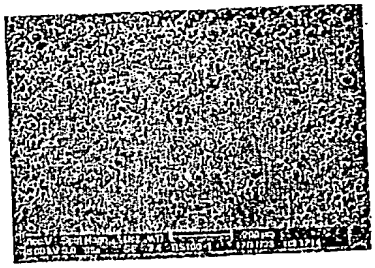


Fig 9A: 3g RS/30mL acetone

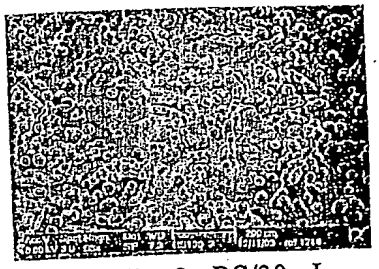


Fig 9B: 3g RS/30mL acetone/ethanol (1:1)

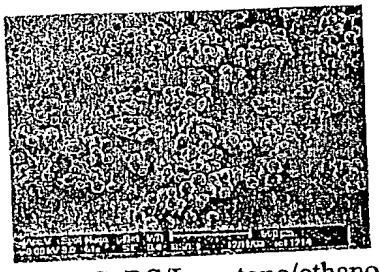


Fig 9C: RS/L acetone/ethanol (2:1)

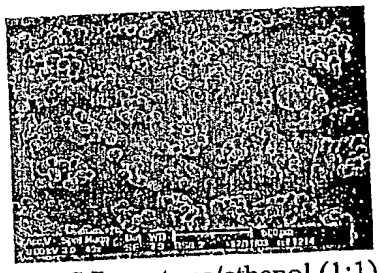


Fig 9D: RS/L acetone/ethanol (1:1)

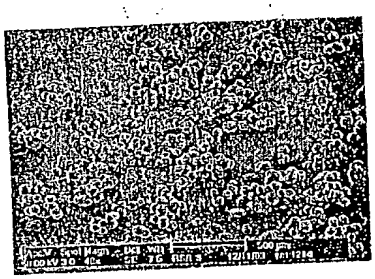


Fig 9E: RS/L acetone/ethanol (1:2)

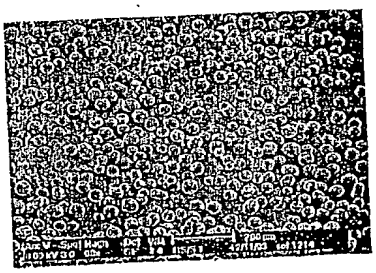


Fig 9F: RS/S acetone/ethanol (2:1)

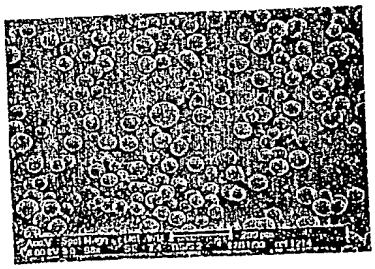


Fig 9G: RS/S acetone/ethanol (1:1)

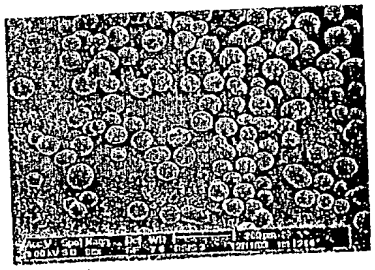
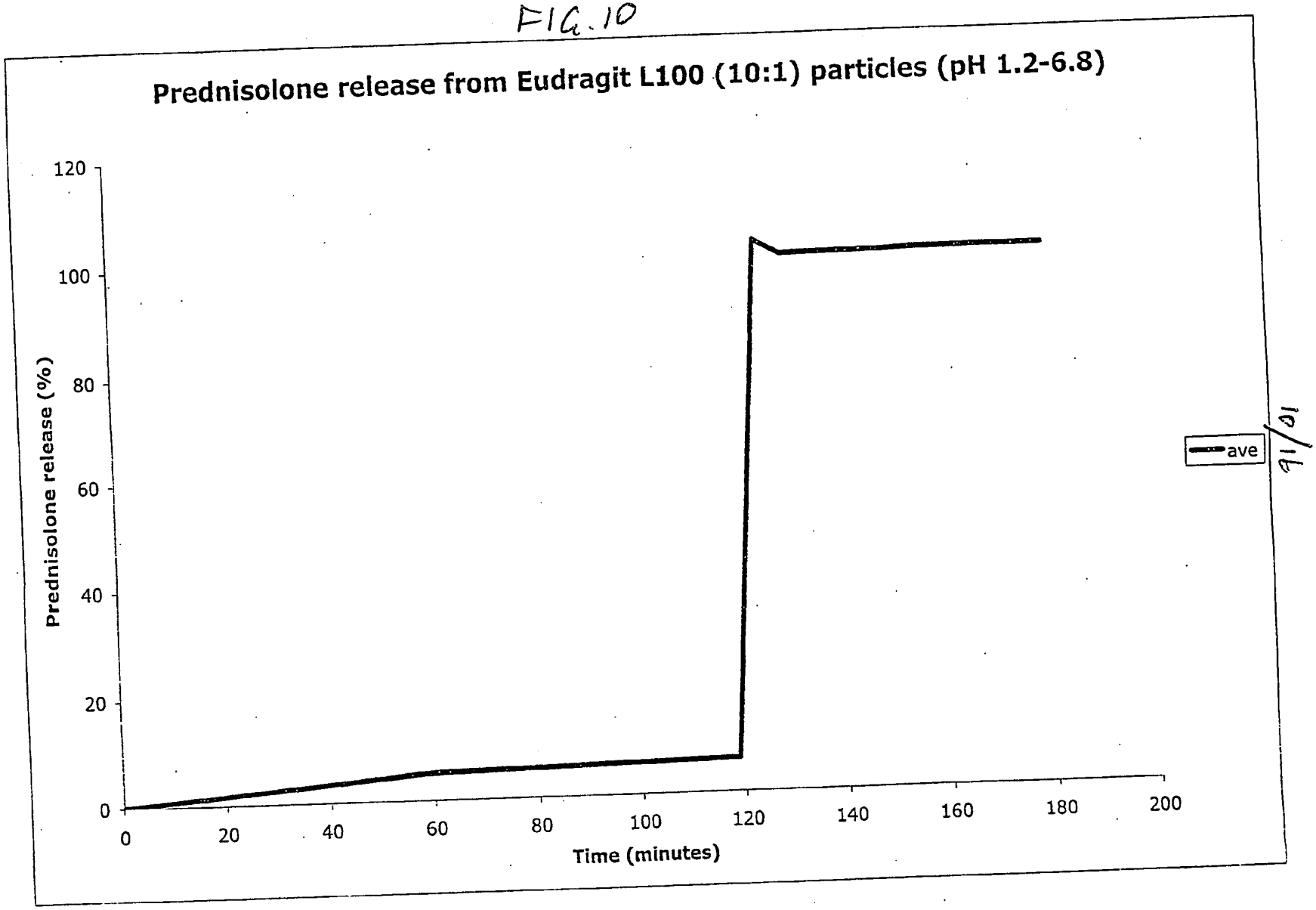


Fig 9H: RS/S acetone/ethanol (1:2)

FIG. 10


Prednisolone release from Eudragit L100 (10:1) particles (pH 1.2-6.8)
Prednisolone release (%)
120
100
80
60
40
20
0
0
20
40
60
80
100
120
140
160
180
200
Time (minutes)
ave

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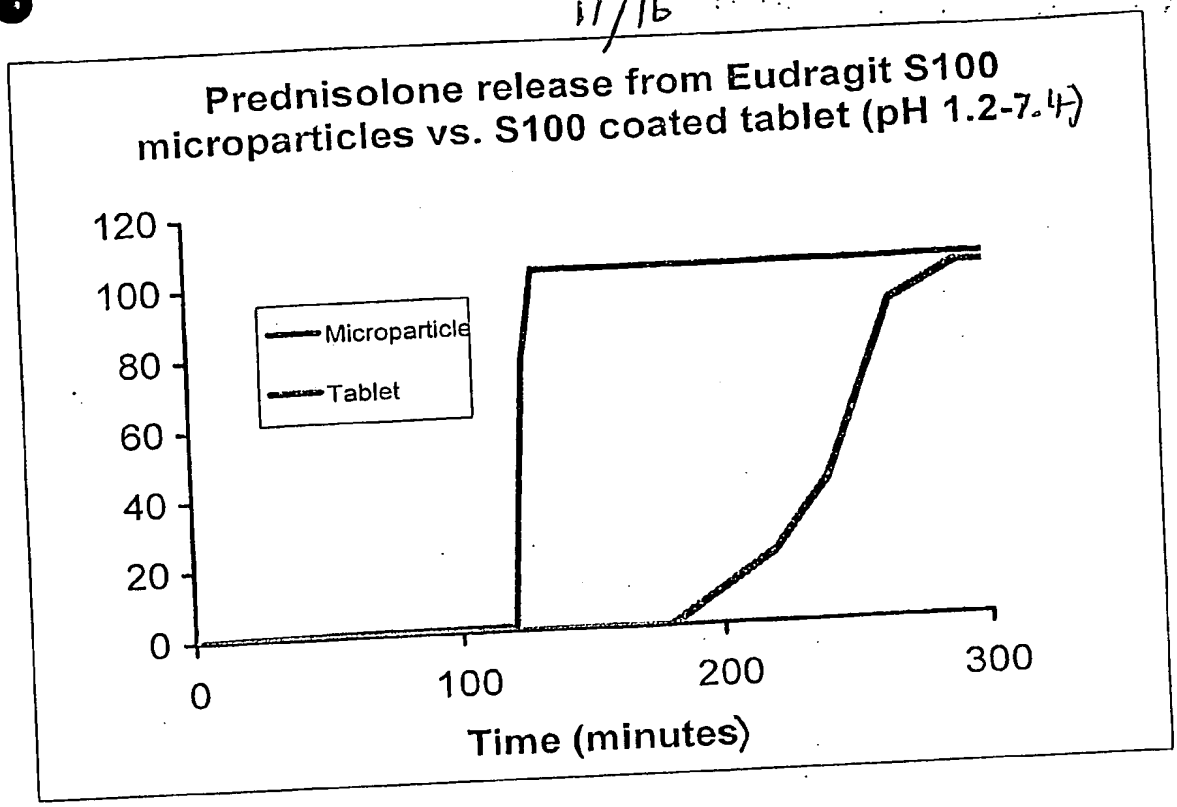
Prednisolone release from Eudragit S100 microparticles vs. S100 coated tablet (pH 1.2-7.4)
120
100
80
60
40
20
0
Microparticle
Tablet
0
100
200
300
Time (minutes)

FIG. 11

FIG. 12

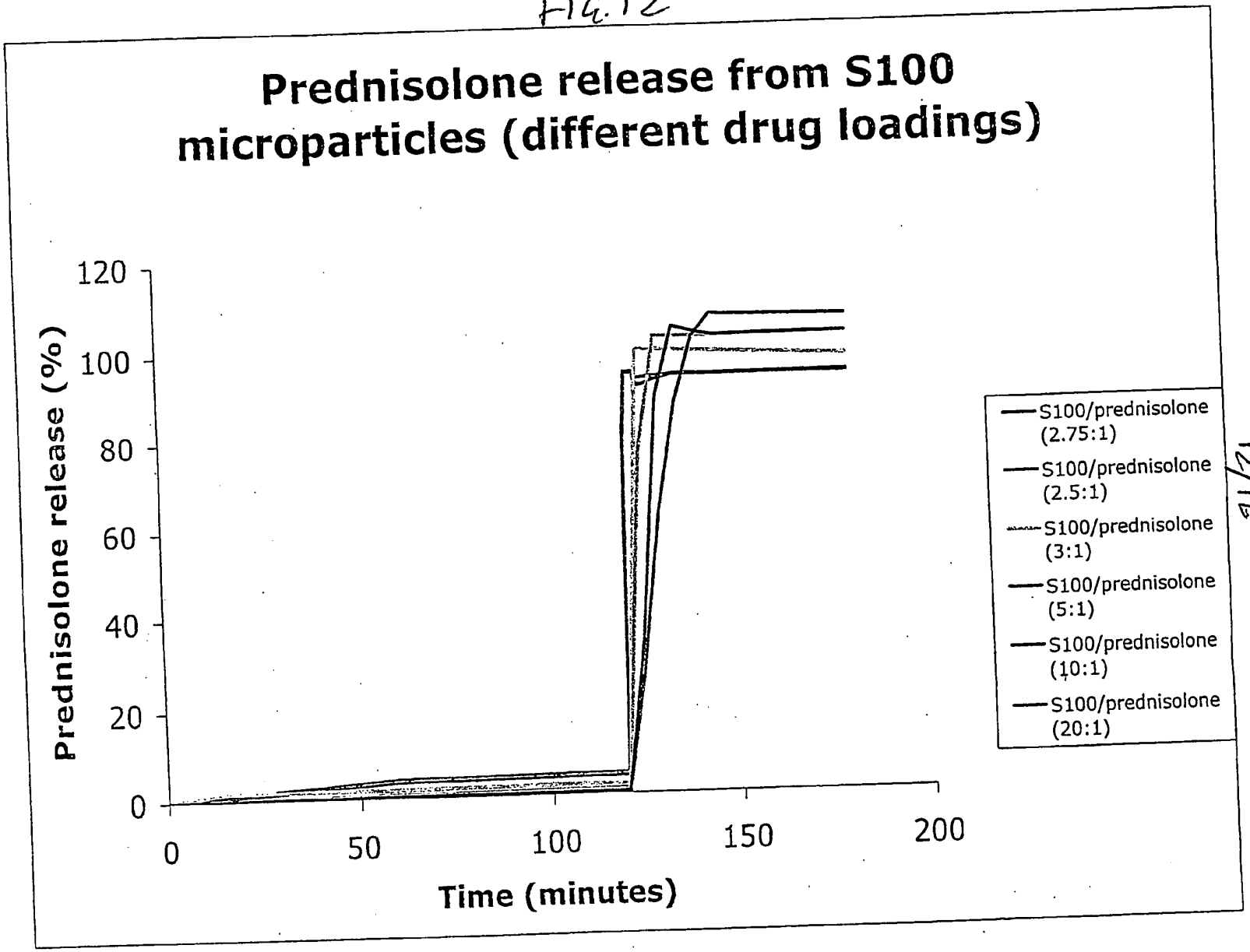
Prednisolone release from S100 microparticles (different drug loadings)
Prednisolone release (%)
120
100
80
60
40
20
0
0
50
100
150
200
Time (minutes)
S100/prednisolone (2.75:1)
S100/prednisolone (2.5:1)
S100/prednisolone (3:1)
S100/prednisolone (5:1)
S100/prednisolone (10:1)
S100/prednisolone (20:1)

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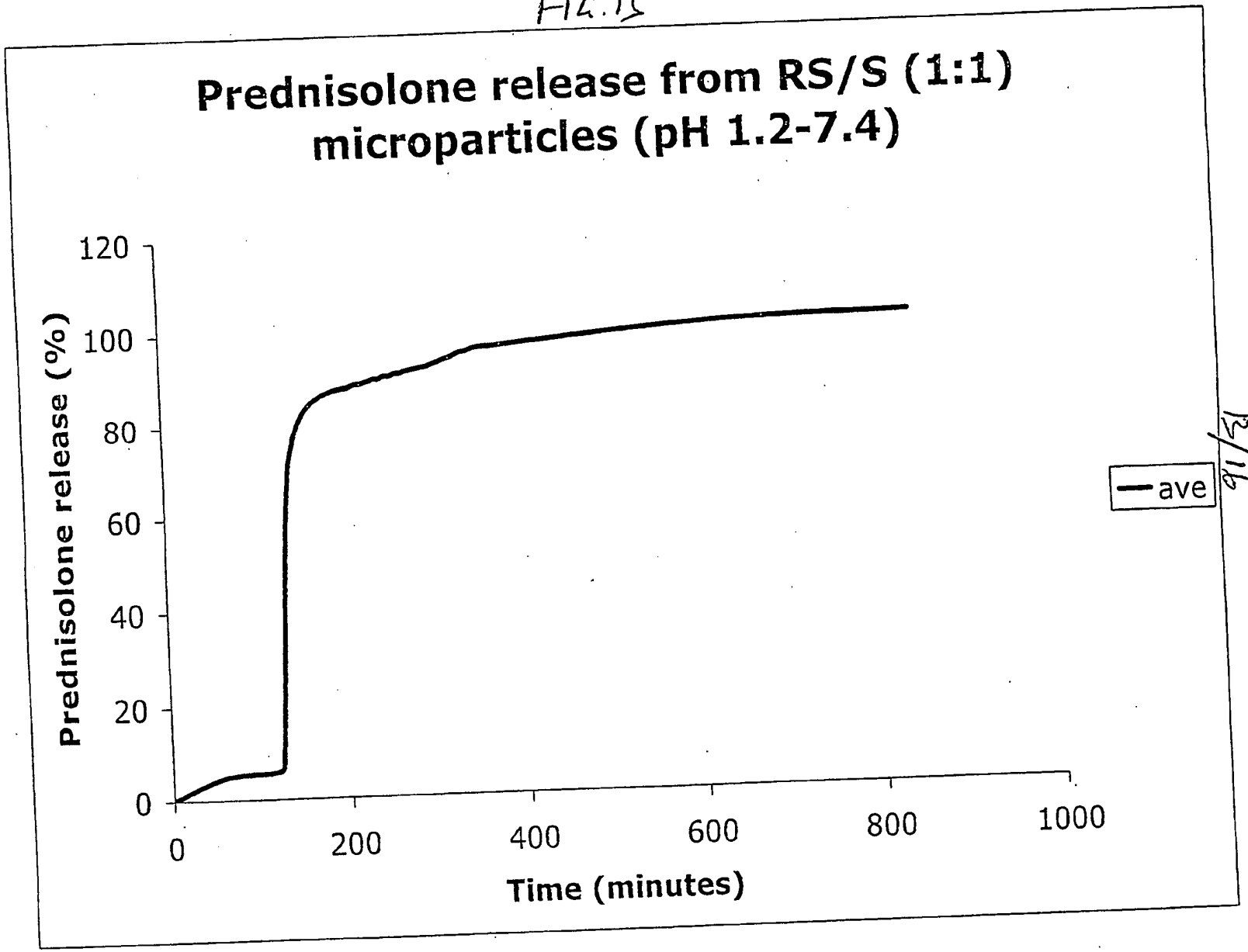
FIG. 13


Prednisolone release from RS/S (1:1) microparticles (pH 1.2-7.4)
Prednisolone release (%)
120
100
80
60
40
20
0
0
200
400
600
800
1000
Time (minutes)
ave

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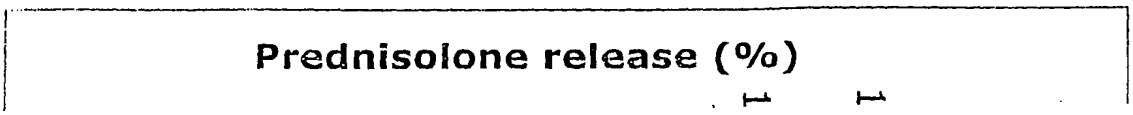
Prednisolone release

(%)


Prednisolone release (%)